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Antioxidant properties, protein binding capacity and mineral contents of some plants traditionally used in the management of animal wounds

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ABSTRACT

Herbal medicines are considered an intricate and integral part of humankind's knowledge systems. Time has proven their efficacy and safety for both human and animal applications. Modern science, guided by indigenous knowledge systems can further optimize the use of various herbal products. To widen the current focus on herbal medicines, a study was carried-out to determine antioxidant properties, phytochemical and mineral contents of some medicinal plants used in ethnoveterinary practices in the management of animal wounds in Zimbabwe. The studied plants were *Cissus quadrangularis* L, *Erythrina abyssinica* Lam. ExDC. and *Adenium multiflorum* Klotzsch. Radical scavenging activities, antioxidant properties were determined using the DPPH and the β-carotene-linoleic acid model while the total phenolic content was determined using the Folin C method, flavonoid content using the aluminium assay and mineral content was investigated using the ICP-OES method. All extracts investigated exhibited radical scavenging activities and antioxidant properties, with *C. quadrangularis* leaf extracts exhibiting superior activities such as radical scavenging (EC_{50} of 21.04 ± 3.00 μg/ml) and antioxidant properties (ORR of 0.03 ± 0.01). Variations were observed in the total phenolic, flavonoid and metal contents. *C. quadrangularis* leaf extracts exhibited highest amounts of total phenolic and flavonoid contents. The *E. abyssinica* (bark) and *A. multiflorum* extracts exhibited moderate (40–70%) affinity for protein binding while the rest of the extracts exhibited high affinity. Their antioxidant properties, phytochemical profile and mineral content justify applications in animal wound management and many other human and/or animal uses.

1. Introduction

Humankind and their communities have benefited, to a large extent, from their indigenous knowledge systems for innumerable years. Such knowledge systems include plants useful for animal and/or human disease management. Several authors have identified many advantages of using herbal medicines including low costs, accessibility, high potency, good tolerance and few side effects among others (Pathak and Jyoti Das, 2013). Several adverse side effects coupled with exorbitant costs of modern pharmaceutical medicines have contributed to the growing use of herbal medicines in the management of both human and animal diseases. Zimbabwe has a very large untapped botanical wealth. It has many similarities with her southern neighbour South Africa which has around 24,000 species comprising more than 10% of the world's vascular plant flora (Germishuizen and Meyer, 2003).

The animal skin and associated membranes makes the largest organ and the first-line of defence. The skin is constantly exposed to infectious organisms and other toxicants (Mishra, 2011). Soyelu and Masika (2009) have defined animal wounds in a manner that includes sores, abscesses, warts and inflamed skin lesions. These forms of tissue damage have several outcomes such as acute inflammation (representing initial response aimed at eliminating dead material and infection minimisation), restitution (i.e. damaged tissue replaced by identical tissue which is ideal), fibrous repair (scar tissue) and/or chronic inflammation (persistent tissue damage). Wounds such as lesions, warts, sores or abscesses may also be complicated with pathogenic microorganisms like bacteria (e.g. *Staphylococcus* species, *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia plymuthica*, *Proteus mirabilis*, *Salmonella* species); fungi (e.g. *Candida albicans*, *Trichophyton mentagrophytes* var. interdigitale, *Aspergillus* species, *Fusarium* species) and even viruses like *Papillomavirus* (Becker et al., 1991; Crutchfield et al., 2005; Alghalibi et al., 2011; Shakoor et al., 2012).

Plants produce a diverse array of secondary metabolites with many functions, such as defence against microbial and viral invasions. Such

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molecules have the ability to inhibit growth of infectious microorganisms, offer good antioxidant and anti-inflammatory properties, and provide rare micro-nutrients and minerals. All the listed molecules possesses wound healing and restitution properties. *Dalbergia nyasae* Bak. f., *Cissus quadrangularis* L., *Adhatoda vasica* Nees, *Annona squamosa* L., *Helianthus annuus* L., *Curcuma amada* Roxb., *Hypericum hookerianum* Wight & Arn., *Sida cordifolia* Linn., *Semecarpus anacardium* Linn., *Coelogyne cristata*, among others are known to help in soft, nervous, bone and muscle tissue repair among other healing powers (Deka et al., 1994; Jaiswal et al., 2004; Shah, 2011). However, there is a limitation of information on the validation of these species in ethnoveterinary studies. Three plant species used in traditional animal and human wound management were selected in the study based on an ethnopharmacological study conducted in some areas of Zimbabwe. Table 1 summarises the traditional human and ethnovetarinary uses of the three plants namely: *Cissus quadrangularis* L., *Adenium multiflorum* Klotzsch and *Erythrina abyssinica* Lam. Ex DC.

The current study was conducted to determine the antioxidant properties, phytochemical and mineral profiles of these three plant species commonly used in the management of animal wounds in Zimbabwe.

2. Materials and methods

2.1. Plant collection and identification

The plant materials were collected from Mberengwa, Midlands Province (*C. quadrangularis*–20°28′09.0″S 29°55′23.3″E.), Karoi, Mashonaland West Province (*E. abyssinica* – 16°49′44.1″S 29°41′19.8″ E) and Buhera, Manicaland Province (*A. multiflorum* – 19°17′10.7″S 31°25′20.2″E) of Zimbabwe during the months of October – December 2014. Species identification was done by qualified botanists from the National Herbarium and Botanic Garden and University of Zimbabwe, Harare, Zimbabwe where specimens were submitted.

2.2. Preparation and extraction

Fresh leaf and stem samples from *C. quadrangularis*, whole plant of *A. multiflorum* and leaf, bark samples of *E. abyssinica* were separately oven dried at 50 °C for 48 h. Dried plant materials were ground into powders and extracted (1:20 w/v) with 50% aqueous methanol in an ultrasonic bath for 1 h. The extracts were filtered under vacuum through Whatman's No. 1 filter paper. The extracts were then concentrated under pressure using a rotary evaporator at 30 °C and completely dried under a stream of air. Fresh extracts of 50% aqueous methanol were used in the phytochemical analysis while the dried ones were dissolved in 50% methanol to determine concentrations for the antioxidants assays.

2.3. Bioassays: Antioxidant activity and phytochemical levels

2.3.1. DPPH radical scavenging activity

The DPPH radical scavenging assay was done as described by Karioti et al. (2004), with relevant modifications. Briefly, 15 μl of each plant extract diluted with methanol were added to a methanolic DPPH solution to give a final volume of 1.5 ml. The concentration of DPPH in the resultant final reaction was 50 μM. The reaction mixtures were prepared under dim light conditions and incubated at room temperature for 30 min. The decreases in the purple colouration of the reaction mixtures were read using a spectrophotometer at 517 nm. Standard antioxidant i.e. ascorbic acid (e.g. 5, 10, 20, 40, 80 μM) solutions were used as positive controls. Solutions with the same chemicals except for the extracts or standard antioxidants were used as negative controls. Methanol was used to blank the spectrophotometer. The background correction was done by subtracting the absorbance of the extracts without DPPH. Each test was done in triplicate. The free radical scavenging activity (RSA) as determined by the decolouration of the DPPH solution was calculated according to the formula;

$$\text{RSA}\ (\%) = \left\{1 - \left(\frac{\text{Abs}_{517\ \text{nm}}\ \text{Sample}}{\text{Abs}_{517\ \text{nm}}\ \text{Neg Control}}\right)\right\} \times 100,$$

where Abs_{517} sample is the absorbance of the reaction mixture containing the extract or positive control solution and Abs_{517}Neg control is the absorbance of the negative control (Karioti et al., 2004). Radical scavenging activity (%) was plotted against the extract concentration. The EC_{50} values, representing the amount of extract required to decrease the absorbance of DPPH by 50% were calculated from the logarithmic non-linear regression curve.

2.3.2. β-carotene-linoleic acid model system (CLAMS)

The delay or inhibition of β-carotene and linoleic acid oxidation was measured according to the method described by Amarowicz et al. (2004) and Ndhlala et al. (2014) with minor modifications. β-carotene (10 mg) was dissolved in 10 ml chloroform in a brown Schott bottle. The excess chloroform was evaporated under vacuum, leaving a thin film of β-carotene. Linoleic acid (200 μl) and Tween 20 (2 ml) were immediately added to the thin film of β-carotene and mixed with aerated distilled water (497.8 ml), giving a final β-carotene concentration of 20 μg/ml. The mixture was further saturated with oxygen by vigorous agitation to form an orange coloured emulsion. The emulsion (4.8 ml) was dispensed into test tubes to which 200 μl of the resuspended plant extracts at 6.25 mg/ml or butylated hydroxytoulene (BHT) (6.2 5 mg/ml) were added, giving a final concentration of 250 μg/ml in the reaction mixtures. Absorbance for each reaction was immediately ($t = 0$) measured at 470 nm and incubated at 50 °C, with absorbance of each reaction mixture being measured every 30 min for 180 min. Tween 20 solution was used to blank the spectrophotometer. The negative control consisted of 50% methanol in place of the sample. The rate of β-carotene bleaching was calculated using the following formula;

$$\text{Rate of bleaching (R)} = \left\{\ \ln\left(\frac{A_{t=0}}{A_{t=t}}\right)\right\} \times \frac{1}{t}$$

where $A_{t=0}$ is the absorbance of the emulsion at 0 min; and $A_{t=t}$ is the absorbance at time t. The calculated average rates were used to determine the antioxidant activity (ANT) of the respective herbal

Table 1
Traditional uses of the three plant species in ethnoveterinary medicine related to wounds management.

Family name	Scientific name	Common name	Traditional uses	Ref
Apocynaceae	*Adenium multiflorum* Klotzsch	Impala Lily- Eng; *Chisvosve* - Shn	Used to treat warts, calluses and other hard inflamed areas of the skin	Van Wyk and Gericke (2000)
Fabaceae	*Erythrina abyssinica* Lam. Ex DC.	Uganda coral, erythrina, flame tree, lucky bean tree – Eng; Munhimbiti, Mutiti - Shn	Used in the management of topical inflammation and infections especially involving mucus membranes as in eyes	Orwa et al. (2009)
Vitaceae	*Cissus quadrangularis* L.	veldt grape or devil's backbone - Eng; *Muvengahonye* - Shn	Used in traditional medicine to treat skin infections, burns and wounds as well as heal broken bones and injured ligaments and tendons in animals	Bharti et al. (2014)

Eng - English; Shn - Shona.